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Wheat streak mosaic virus genotypes introduced to Argentina are closely related to isolates from the American Pacific Northwest and Australia

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BRIEF REPORT

Wheat streak mosaic virus genotypes introduced to Argentina are closely related to isolates from the American Pacific Northwest and Australia

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Abstract *Wheat streak mosaic virus* (WSMV) was first detected in Argentina in 2002. Comparison of 78 WSMV coat protein sequences revealed that three Argentine isolates were closely related to isolates from the American Pacific Northwest (APNW) and Australia. Complete sequences were determined for one Argentine isolate, four APNW isolates, and three additional isolates from other regions of the USA. Comparison of these eight new sequences with five previously sequenced isolates of WSMV confirmed close affinity of WSMV from Argentina with APNW isolates. Collectively, these results indicate concurrent establishment of the same WSMV lineage in both Argentina and Australia.

Wheat streak mosaic virus (WSMV) is the type species of the genus *Tritimovirus* in the family *Potyviridae* [17]. First reported from the Central Great Plains of the USA in the 1920s [12], WSMV is widely distributed in wheat-growing regions of North America and Eurasia. Previous phylogenetic analyses [14, 18] grouped WSMV isolates into four distinct clades. Clade A, represented by the El Batán 3 isolate from Mexico [15], differs in nucleotide sequence from the other three clades by ~20% [1]. WSMV isolates from Central Europe and Russia [14] comprise Clade B and differ in nucleotide sequence from Clade C and D genotypes by ~10%. An isolate from Iran [14] represents Clade C, differing in sequence from Clade D genotypes by ~8%. Numerous WSMV isolates from temperate North America belong to Clade D and share >96% nucleotide sequence identity. Clade D also includes two isolates from Turkey, suggesting intercontinental movement at some time in the past [14].

WSMV in Australia was first detected in greenhouse-grown wheat germplasm in 2002 and soon thereafter was found in several Australian wheat-growing regions [3]. Analysis of 3′-proximal partial nucleotide sequences [2] indicated that Australian isolates of WSMV were closely related to each other, and also to certain Clade D isolates from the American Pacific Northwest (APNW). As several of the Australian isolates are seed borne in wheat at low frequency [2, 11], infected seed has been implicated as the means of initial introduction. Subsequent spread of WSMV within Australia was likely due to both seed transmission and vector transmission by the wheat curl mite [8].

In contrast to the establishment of WSMV in Australia, introduction of WSMV to Argentina is less well documented. WSMV was first detected in Argentina in 2002 [19] by symptom expression in field grown wheat in the Cordoba province; identity of the virus was verified by reverse transcription-polymerase chain reaction (RT-PCR). However, the relationship of WSMV genotypes from Argentina with those occurring elsewhere in the world is unknown. Here, we report phylogenetic placement of three Argentine isolates of WSMV, based on coat protein (CP) gene sequences. Subsequently, we confirmed phylogenetic placement of WSMV from Argentina via complete sequence comparisons of 1 Argentine isolate with 12 completely sequenced WSMV isolates (7 of which are reported for the first time here) from the USA, Mexico, and Eurasia.

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The origins of WSMV isolates used in sequence comparisons are listed in Table 1. Total nucleic acid samples for Argentine isolates Arg1, Arg2, and Arg3 were extracted [13] from systemically infected wheat leaves (~2 g). Complementary DNA was synthesized by RT using the primer RCF1 (5′-AGCTGGATCCTTTTTTTTTTTTTT-3′) [13]. A ~1,267-bp fragment was amplified by PCR using the primers XV1 (5′-GATTCCGTTGAAGGATTTGTA ACTT-3′) and XC1 (5′-AACCCACACATAGCTACCA AG-3′) [6]. The amplified products correspond to WSMV-Sidney 81 nts 8,105–9,371 [17], encompassing the entire CP coding region (nts 8,189–9,238) and flanked upstream by the 3′-proximal portion of the nuclear inclusion b coding region and downstream by 3′-untranslated sequences. The amplicons were gel purified and cloned into pGEM-Teasy (Promega, Madison, WI). For each Argentine isolate, a consensus sequence was generated based on three independent clones per isolate.

Complete CP gene nucleotide sequences for 78 WSMV isolates (Table 1) and an isolate of *Oat necrotic mottle virus* (ONMV; GenBank accession number AY377938), the most closely related tritimovirus species [16], were aligned using CLUSTAL X. Neighbor-joining analysis was performed using CLUSTAL X with 1,000 bootstrap iterations; gaps were excluded from the analysis, and all other parameters were set to default values. The resulting tree (Fig. 1) was visualized using the TreeView program, with all nodes having less than 60% bootstrap support collapsed to polytomies, and rooted with ONMV designated as the outgroup. CP gene sequences of the three Argentine isolates clustered in Clade D. Although Clade D is mostly unstructured, significant bootstrap support existed for at least four subclades ('Sidney', 'Type', 'APNW', and a fourth lacking a single proline codon and including isolate H95S) as described previously [18]. Interestingly, the three Argentine isolates shared a node with high bootstrap support (>86%) in common with and exclusive to 15 Australian isolates and four APNW isolates (ID96, ID99, WA94, and WA99). It should be noted that the positions of ID99 and Mon96 in the present analysis (Fig. 1) are reversed (e.g., ID99 and not Mon96 is part of the APNW subclade) relative to the tree presented previously [18], as these two nucleotide sequences were mutually mislabeled for GenBank submission and in the original analysis of 2002. The GenBank accessions for these two isolates have since been corrected.

To verify relationships inferred by neighbor-joining analysis of CP sequences presented in Fig. 1, the same data set was evaluated using the maximum-likelihood method. A tree was generated using Treefinder with the HKY model of nucleotide substitution and 200 bootstrap replicates [10]. The topology of the CP tree generated by maximum likelihood was identical to the neighbor-joining tree of Fig. 1 with respect to isolates from Argentina, Australia, and the APNW sharing a common node (bootstrap value = 86%) exclusive of all other taxa examined (data not shown).

To further confirm phylogenetic placement of WSMV from Argentina, the complete sequences of one Argentine isolate (Arg2) and seven isolates from the USA were determined. The American isolates were selected for complete sequencing based on known affinity with the APNW subclade (ID96, ID99, WA94 and WA99), a representative member of the subclade sharing a single proline codon deletion (H95S), and two arbitrary draws (H98 and Mon96) from the unstructured portion of clade D. Australian isolates were not available for complete sequencing. For each isolate, total nucleic acid samples were extracted as described above. RT was conducted using a mixture of RCF1 and random hexamer primers. Complementary DNA was used as template in PCR with the following primer sets to amplify each genome in overlapping "thirds" corresponding to WSMV-Sidney81 nucleotide coordinates: nts 1–3618 (5′-GGA TCCATTTAGGTGACACTATAG**AAATTAAACCAAC CCAAATC**-3′ and 5′-CCGGATCC**TATTCAACCAATT C**-3′), nts 2,339–6,691 (5′-CCGGATCC**GGGTTCCAAG AGACTGTT**-3′ and 5′-GACTTCTAGATCA**TTGCCAA CTAACCAAG**-3′), and nts 5,414–9,384 (5′-GTCTAA GCTTG**GGCAAAGCAGCACGCA**-3′ and RCF1), with 3′-proximal nucleotides in bold corresponding to WSMV-Sidney81 plus or minus sense sequences. In cases where no product was amplified with a given primer set, alternative primers were utilized such that overlap among amplified "thirds" of the genome was maintained. PCR products were cloned into pGEM-Teasy and three independent clones were sequenced for each. Consensus sequences for each complete genome were compiled using DNA Sequencher 4.1.

The eight complete nucleotide sequences determined in this study were aligned with five previously sequenced isolates of WSMV (Sidney81, Type, Czech, TK1, and El Batán 3) and the complete genome of ONMV. Neighbor-joining analysis was performed on the aligned complete sequences as described above, and the resulting tree (Fig. 2) was rooted using ONMV as the outgroup; all nodes having less than 60% bootstrap support were collapsed to polytomies. The complete sequence of Arg2 was most closely related to isolate ID96 (98.9% nucleotide sequence identity) and shared a node exclusive to and in common with all four completely sequenced isolates from the APNW. Complete sequences of Australian WSMV isolates were unavailable for comparison.

The 13 WSMV complete sequences also were used to generate alignments of individual gene sequences (P1, HC-Pro, P3, CI, NIa, and NIb) and subjected to neighbor-joining analysis (as described above); ONMV was used as the outgroup. In all cases, tree topology was identical (data

Table 1 Wheat streak mosaic virus (WSMV) isolates used in sequence comparisons

WSMV isolate	Accession number	Geographic Origin	Reference
Sidney81[a]	AF057533	Nebraska, USA	[17]
Type[a]	AF285169	Kansas, USA	[1]
El Batán 3[a]	AF285170	Mexico	[1]
Czech[a]	AF454454	Czech Republic	[14]
Hungary	AF454456	Hungary	[14]
Russia	AF454459	Russia	[14]
Iran	AF454458	Iran	[14]
TK1[a]	AF454455	Turkey	[14]
TK2	AF454457	Turkey	[14]
H95S[a]	AF511614	Kansas, USA	This study[b]
H98[a]	AF511615	Kansas, USA	This study[b]
ID96[a]	AF511618	Idaho, USA	This study[b]
ID99[a]	AF511619	Idaho, USA	This study[b]
Mon96[a]	AF511630	Montana, USA	This study[b]
WA94[a]	FJ348358	Washington, USA	This study
WA99[a]	AF511643	Washington, USA	This study[b]
Arg 1	FJ348356	Argentina	This study
Arg 2[a]	FJ348359	Argentina	This study
Arg 3	FJ348357	Argentina	This study
Mt. Burdett	DQ888801	Australia	[2]
Yerritup	DQ888802	Australia	[2]
Gibson	DQ888803	Australia	[2]
Galong	DQ888804	Australia	[2]
Kondonin	DQ888805	Australia	[2]
SP-5	DQ462276	Australia	[2]
SP-6	DQ462277	Australia	[2]
Tamworth1	AY327866	Australia	Mago et al., unpublished
Tamworth2	AY327867	Australia	Mago et al., unpublished
Adelaide1	AY327868	Australia	Mago et al., unpublished
Adelaide2	AY327869	Australia	Mago et al., unpublished
Canberra	AY327865	Australia	Mago et al., unpublished
MurrayBridge	AY327872	Australia	Mago et al., unpublished
Horsham	AY327871	Australia	Mago et al., unpublished
Bordertown	AY327870	Australia	Mago et al., unpublished
Ger	AJ889242	Germany	Shi et al., unpublished
PV57	AF511595	Kansas, USA	[18]
S81D	AF511596	Nebraska, USA	[18]
CK93	AF511598	Kansas, USA	[18]
CL93	AF511599	Kansas, USA	[18]
CM93	AF511600	Kansas, USA	[18]
CO85	AF511601	Colorado, USA	[18]
CO87	AF511602	Colorado, USA	[18]
EW95	AF511603	Kansas, USA	[18]
FO93	AF511604	Kansas, USA	[18]
GH95	AF511605	Kansas, USA	[18]
GO93	AF511606	Kansas, USA	[18]
GY93	AF511607	Kansas, USA	[18]
H81	AF511608	Kansas, USA	[18]

Table 1 continued

WSMV isolate	Accession number	Geographic Origin	Reference
H88	AF511609	Kansas, USA	[18]
H94PM	AF511610	Kansas, USA	[18]
H94S	AF511611	Kansas, USA	[18]
H94USDA	AF511612	Kansas, USA	[18]
H95LB	AF511613	Kansas, USA	[18]
HM93	AF511616	Kansas, USA	[18]
HV91	AF511617	Kansas, USA	[18]
IHC	AF511620	Canada	[18]
KM93	AF511621	Kansas, USA	[18]
KY00	AF511622	Kentucky, USA	[18]
KY0074	AF511623	Kentucky, USA	[18]
KY0083SV	AF511624	Kentucky, USA	[18]
LC95	AF511625	Kansas, USA	[18]
LG92	AF511626	Kansas, USA	[18]
MO99A	AF511627	Missouri, USA	[18]
MO99B	AF511628	Missouri, USA	[18]
MO00	AF511629	Missouri, USA	[18]
ND	AF511631	North Dakota, USA	[18]
NE96	AF511632	Nebraska, USA	[18]
OK98	AF511633	Oklahoma, USA	[18]
OSU	AF511634	Unknown	[18]
PL95	AF511635	Kansas, USA	[18]
PN95	AF511636	Kansas, USA	[18]
PV106H	AF511637	Ohio, USA	[18]
PV106JM	AF511638	Ohio, USA	[18]
PV91H	AF511639	Kansas, USA	[18]
RO95	AF511640	Kansas, USA	[18]
SD96	AF511641	South Dakota, USA	[18]
TX96	AF511642	Texas, USA	[18]
WO93	AF511644	Ohio, USA	[18]

[a] Complete nucleotide sequence

[b] 3′-proximal sequences reported in [18]

not shown) to that of Fig. 2 with respect to isolate Arg2 sharing a node in common with and exclusive to the four APNW isolates. Bootstrap values (based on 1,000 iterations) for the basal node of the Arg2/APNW lineage were 910 (P1), 842 (HC-Pro), 673 (P3), 994 (CI), 982 (NIa), and 718 (NIb).

WSMV isolates from Argentina, Australia, and the APNW clearly constitute a single lineage (subclade) within Clade D. Limited sequence variation among the Australian and Argentine isolates was observed. This is not unexpected as separate introductions to geographically isolated regions should result in founder effect bottlenecks followed by population expansion. Furthermore, variation within each recently established population is not surprising as WSMV lineages have been shown to accumulate independent changes in consensus sequence upon passage from plant to plant [7]. Given that most changes in consensus sequence of a WSMV isolate upon passage are stochastic, due to small effective population sizes resulting from bottlenecks during systemic movement and plant-to-plant transmission [4, 5, 7], variation encountered within and between the Argentine and Australian populations likely reflects genetic drift rather than differential selection.

Why has this same WSMV lineage recently invaded two isolated wheat-growing regions on separate continents? Given that Clade D is a large polytomy with 38 branches at the basal node (Fig. 1), it is unlikely that two random draws from Clade D would share the same branch. There are two non-exclusive explanations for the recent intercontinental dispersion of this lineage: (1) It was introduced from a common source and/or (2) this particular lineage is seed borne at a rate greater than that of other WSMV

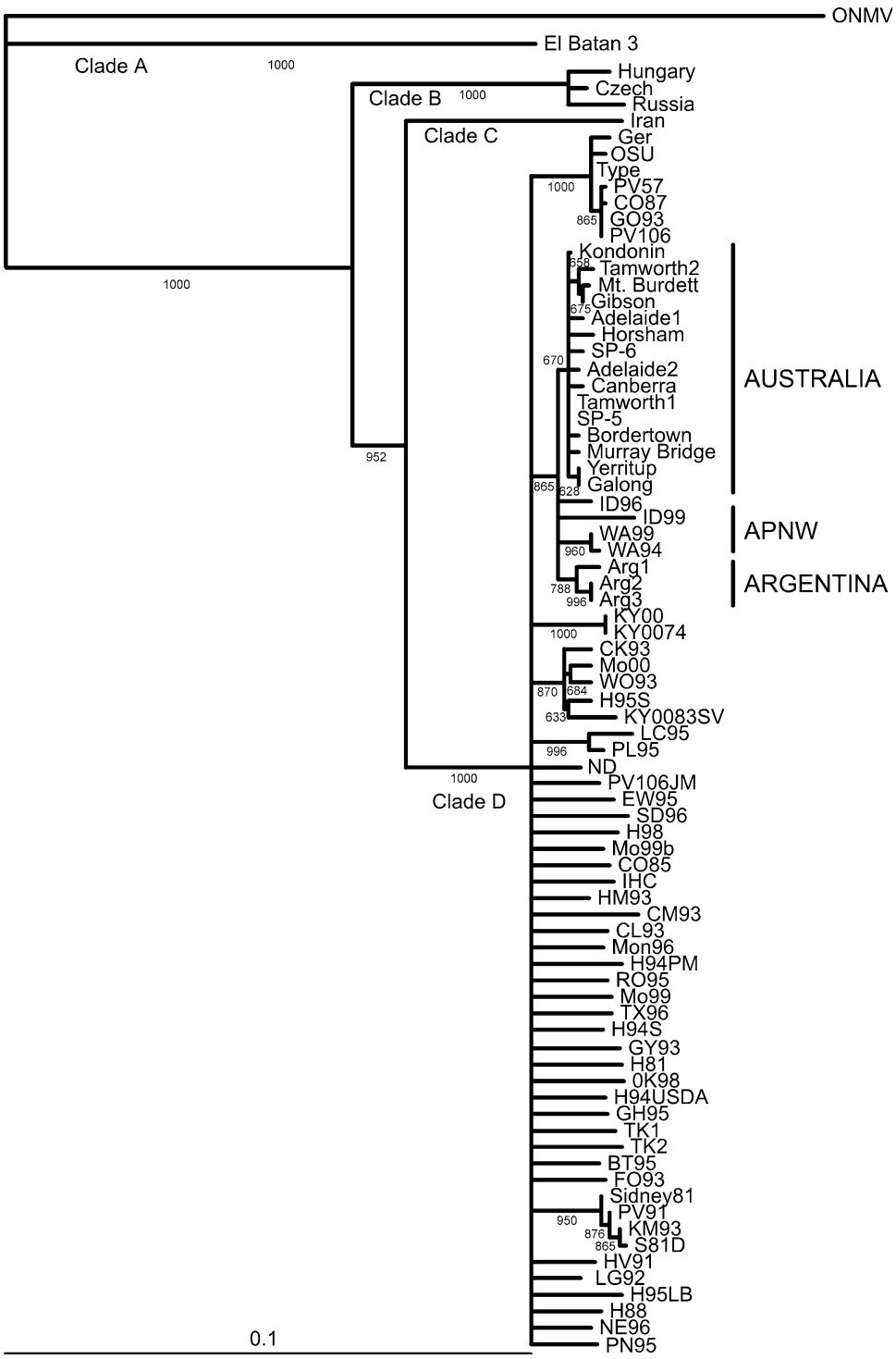

Fig. 1 Phylogenetic relationships among coat protein (CP) gene nucleotide sequences of 78 wheat streak mosaic virus (WSMV) isolates. Presented is a neighbor-joining tree based on 1,000 bootstrap iterations and rooted with the CP sequence of oat necrotic mottle virus (ONMV) designated as the outgroup. Bootstrap values for nodes with high support (≥60%) are indicated on branches basal to each node; nodes bearing <60% bootstrap support were collapsed to polytomies. WSMV Clades A–D are labeled at the respective basal branch defining each clade. WSMV isolates from the American Pacific Northwest (APNW), Australia, and Argentina shared a common node exclusive of all other taxa and are labeled on the right. Branch lengths are proportional to genetic distance; length of scale bar at lower left corresponds to a genetic distance of 0.1

lineages. With respect to the first explanation, the lineage in question has been present in the APNW since at least 1994 (the earliest year of collection of isolates belonging to the lineage), 8 years before detection of WSMV in Australia and Argentina. However, the first isolations of WSMV in Australia were from greenhouse-grown wheat breeding lines derived from Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) in Mexico [3]. Furthermore, absence of data does not demonstrate that this lineage was not present in other locations that could have served as a source. With respect to the second explanation, WSMV isolates from Australia are seed borne at 0.5–2% [2, 11], a rate several log units greater than that previously reported for WSMV in maize [9]. However, as other

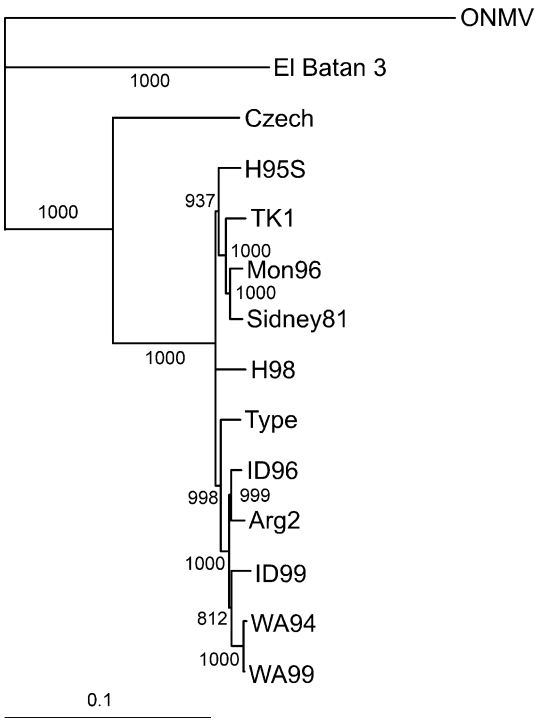

Fig. 2 Phylogenetic relationships among complete nucleotide sequences of 13 wheat streak mosaic virus (WSMV) isolates. Presented is a neighbor-joining tree based on 1,000 bootstrap iterations and rooted with the complete sequence of oat necrotic mottle virus (ONMV) designated as the outgroup. Bootstrap values for nodes with high support (≥60%) are indicated on branches basal to each node; nodes bearing <60% bootstrap support were collapsed to polytomies. Branch lengths are proportional to genetic distance; length of scale bar at lower left corresponds to a genetic distance of 0.1. Note that the WSMV isolate from Argentina (Arg2) shared a node exclusive to and in common with four isolates (ID96, ID99, WA94, and WA99) from the American Pacific Northwest

lineages of WSMV have not been evaluated for seed transmission, it remains unclear whether a rate of ~1% is unusual. Thus, while both explanations are plausible, neither can be validated or excluded at this time.

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